Supporting Information of

"On-surface aggregation of α-Synuclein at nanomolar concentrations results in two distinct growth mechanisms"

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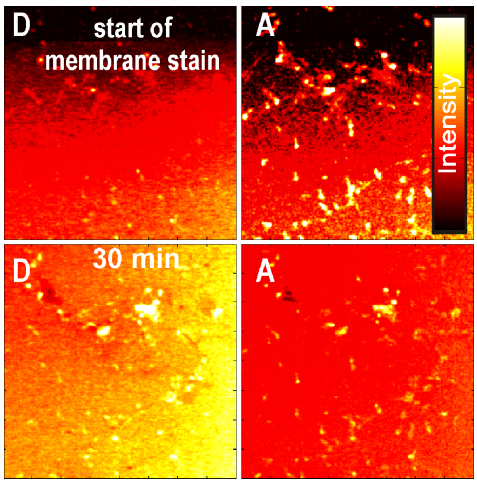

Figure S1. Membrane staining. Subsequent to the experiment presented in Fig. 1 an aqueous solution of the membrane intercalating fluorescent dye CellMask™ was exposed to the surface. The strong increase in fluorescence intensity in both images, donor (D) and acceptor (A), proves that the lipid bilayer was not removed in the course of the experiment. The fluorescence intensity increase of the acceptor channel results from the bleed through that amounts to approximately 6% of the donor intensity (scales are individually adapted).

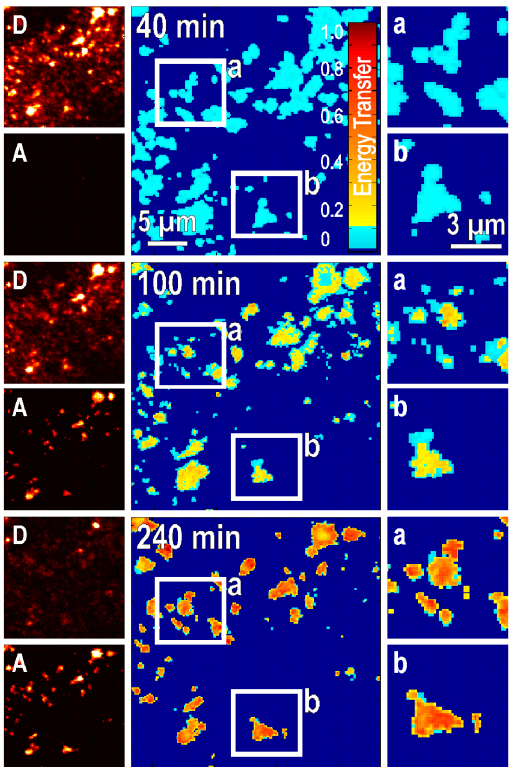

Figure S2. Sequential FRET experiment showing the beginning of α-Syn aggregation on a glass surface at 1 nM total concentration (1×PBS). Firstly, only donor labeled α-Syn was applied to the surface (top row) followed by applying only acceptor labeled α-Syn to the bulk (middle and bottom row). In the FRET images red color indicates high energy transfer rates attributed to aggregation between donor and acceptor-labeled α-Syn, light blue color indicates no energy transfer, i.e. only donor fluorescence.

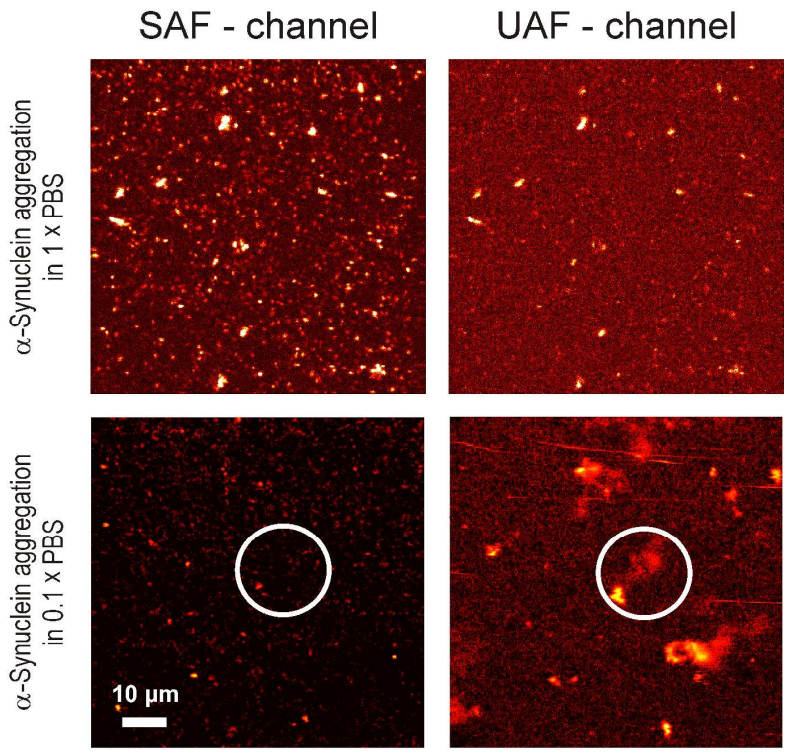

Figure S3. Comparison of α-Syn aggregates grown in high (top row) and low (bottom row) ionic strength conditions. As a representative example of a repeatedly observed feature it is shown that at low ionic strength conditions (16.6 mol/L) large aggregated structures protruding deep into the bulk solution are visible. These appear as 'clouds' in the UAF-channel (bottom right). Such a massive outgrowth is not observed at high ionic strength conditions (166 mol/L). The images are raw images and were recorded after an incubation time of ~16 hours. In both cases a mixed sample composed of 50% donor (DY647) and 50% acceptor (Cy7) labeled α-Syn is applied.

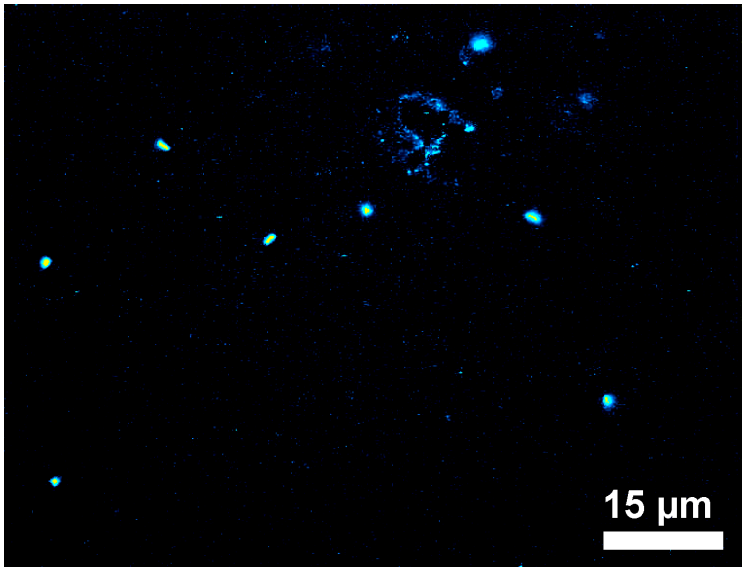

Figure S4. Epifluorescence microscopy images of ThT stained α-Syn aggregates (1 nM, 0.1×PBS on a SLB) at an early stage of the aggregation process. Most aggregates observed on this image exhibit elongated compact structures similar to the type 1 aggregates observed by SAF imaging between 3 and 6 hours of incubation. The positive response to ThT staining indicates that also type 1 aggregates contain amyloid-like structures.

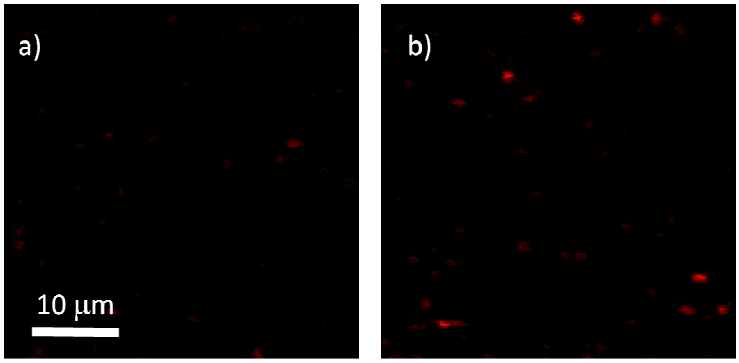

Figure S5. Detection of α-Syn aggregates by amyloid specific antibodies. Amyloid specific OC antibodies raised in rabbit was used to specifically bind to amyloid fibrils (courtesy of Prof. Glabe, University of California, Irvine). The antibodies were diluted to 1:250 in 10% normal goat serum (NGS) in PBS, and introduced to the SLBs via the flow cell after 24 hours of α-Syn incubation (10 nM). After 30 min OC antibody incubation, the SLBs were washed and visualized by incubating the surface with Alexa-647 labeled secondary antibody (1:200 dilution). The locally confined fluorescence signals shown in (b) indicate the presence of amyloid structures whereas the control experiment in (a) shows only unspecific binding of the secondary antibody (no OC antibody present).

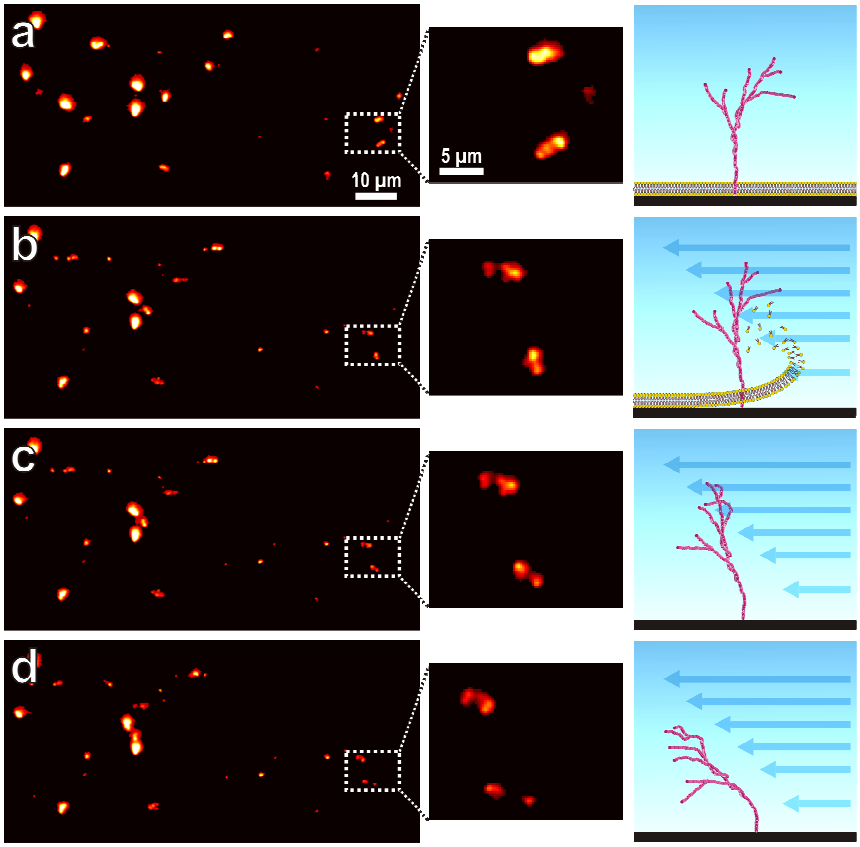

Figure S6. Extended α-Synuclein aggregates grown on a supported lipid bilayer are not rinsed away upon treatment with detergent solution (2% SDS). (a) Bright areas in the microscope image show extended α-Synuclein aggregates protruding into the bulk solution before applying detergent. (b) When detergent solution is applied the supported lipid bilayer is removed (not visible). However, almost all aggregates observed in image (a) are still present in image (b). Images (c) and (d) (especially magnified sections) show that the detergent loosens the 3-dimensional stability of the aggregates which are consequently increasingly stretched by the shear force of the buffer flow.

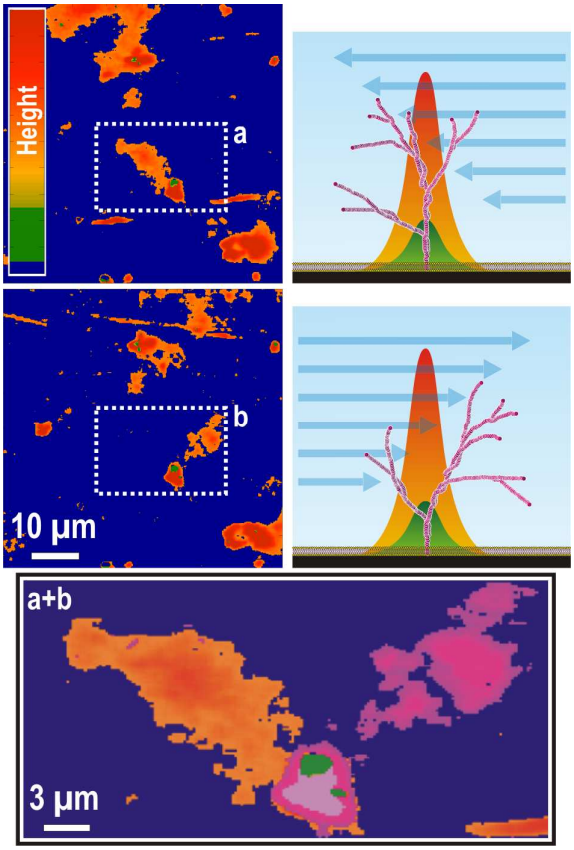

Figure S7. Bending of extended amyloid fibrils in the buffer flow upon of α-Syn aggregation on the glass surface. Scan images were recorded with the buffer flowing from right to left (up) and flowing from left to right (middle) which moves the fibrils protruding deep into the solution. The bottom images represent overlays of the marked regions with the orange color referring to a buffer flow from right to left and the violet color referring to a buffer flow from left to right. The surface anchoring points (green) do not move. An analogous experiment as presented here was conducted on a SLB (Figure 6 in the main text).